Table ~~M~~1. Growth Factor Purification

Purification step	Protein (mg)	Total activity (units)*	Specific activity (units/mg)
Conditioned medium (10 liters)	1.4×10^{3a}	2.5×10^{4}	1.8×10^{1}
Ultrafiltration (retentate)	1.3×10^{3a}	3.2×10^{4}	2.5×10^{1}
HSAC 0.6 MM NaCl pool	0.73^{b}	1.6×10^{4}	2.2×10^{4}
TSK-G3000 SW	8.4×10^{-3b}	2.7×10^{3}	3.2×10^{5}
C_4-HPLC	6.1×10^{-3b}	2.1×10^{2}	3.4×10^{4}

Recoveries were calculated by assuming that all of the mitogenic activity in the starting material was due to the isolated factor.

* One unit of activity is defined as half of the maximal stimulation of thymidine incorporation induced by TSK-purified factor in the BALB/MK bioassay, in which approximately 3 ng of the TSK-purified factor stimulated 1 unit of activity.

[a] Protein was estimated by using the Bradford reagent from BioRad (~~I-23~~). Bradford, M., 1976, Anal. Biochem. 72, 248-254

[b] Protein was estimated by using $A_{214}^{1\%} = 140$.

Table I-2. Target Cell Specificity of Growth Factors

Growth Factor	Epithelial			Fibroblast	Endothelial
	BALB/MK	BS/589	CCL208	NIH/3T3S	Human saphenous vein
KGF	500-1000	2-3	5-10	<1	<1
EGF	100-200	20-40	10-30	10-20	n.d.
TGFa	150-300	n.d.	n.d.	10-20	n.d.
aFGF*	300-500	2-3	5-10	50-70	5
bFGF	100-200	2-3	2-5	50-70	5

Comparison of maximal thymidine incorporation stimulated by KGF and other growth factors in a variety of cell lines, expressed as fold stimulation over background.

This data represents a summary of four different experiments.

* Maximal stimulation by aFGF required the presence of heparin (Sigma), 20 μg/ml.

n.d. = not determined.

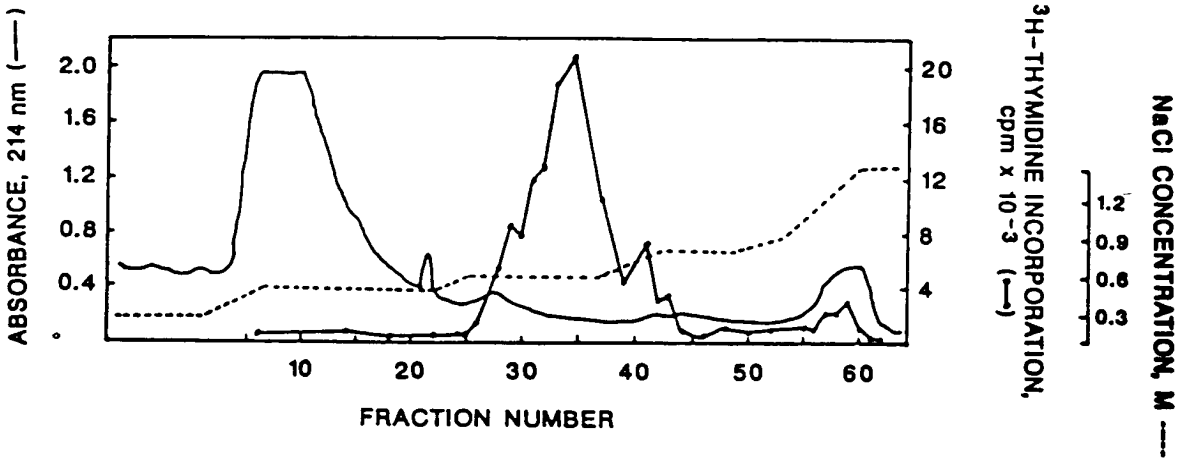


Figure I-1. Heparin-Sepharose affinity chromatography of conditioned medium from M426 human embryonic fibroblasts. Approximately 150 ml of ultrafiltration retentate derived from five liters of M426 conditioned medium were loaded onto a heparin-Sepharose column (6 ml bed volume) in 1 hr. After washing the column with 150 ml of the equilibration buffer, 20 mM Tris-HCl, pH 7.5/0.3M NaCl, the retained protein (<5% of the total protein in the retentate) was eluted with a modified linear gradient of increasing NaCl concentration. Fraction size was 3.8 ml and flow rate during gradient elution was 108 ml/hr. Two µl of the indicated fractions were transferred to microtiter wells containing a final volume of 0.2 ml for assay of ^{3}H-thymidine incorporation in BALB/MK cells as described in the Methods.

Figure I-2. ~~SEE LEGEND NEXT PAGE~~

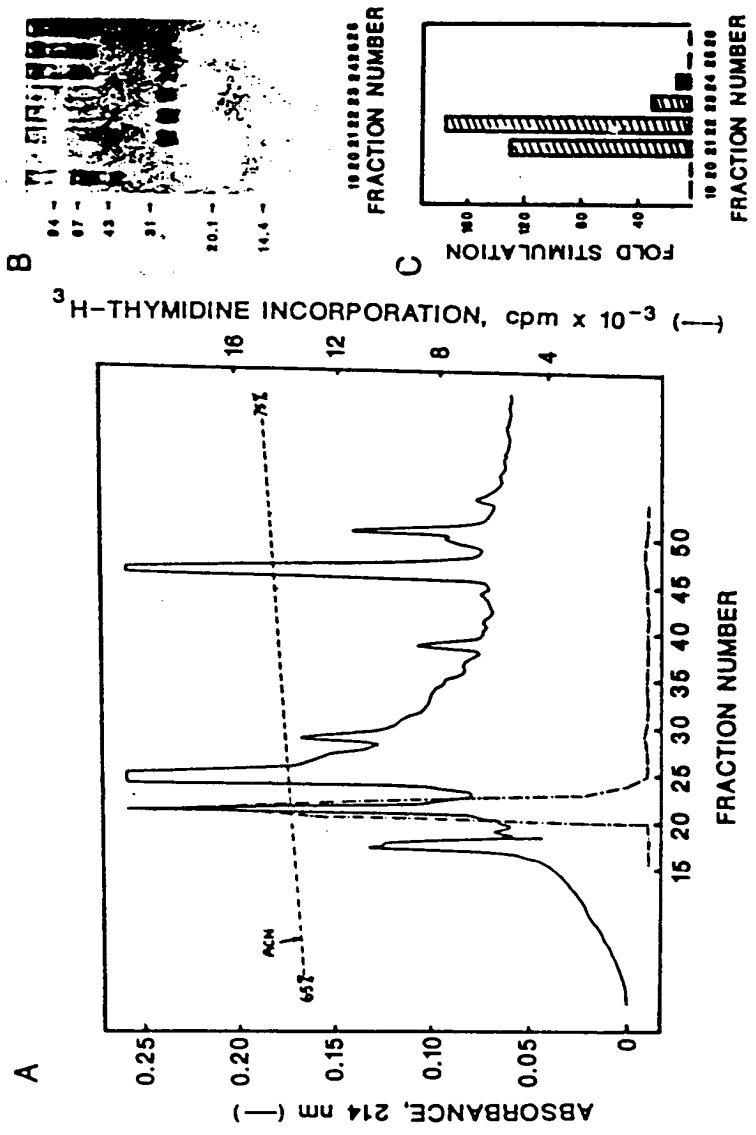


Figure I-2. (A) Reversed-phase C_4 HPLC of BALB/MK mitogenic activity. Active fractions eluted from heparin-Sepharose with ~~0-6M~~ 0.6M NaCl were processed with the Centricon -10 and loaded directly onto a C_4 Vydac column (4.6 x 250 mm) which had been equilibrated in 0.1% trifluoroacetic acid/20% acetonitrile (ACN). After washing the column with 4 ml of equilibration buffer, the sample was eluted with a modified linear gradient of increasing % ACN. Fraction size was 0.2 ml and flow rate was 0.5 ml/min. Aliquots for the assay of ^{3}H-thymidine incorporation in BALB/MK cells were promptly diluted 10-fold with 50 µg/ml bovine serum albumin/20 mM Tris-HCl, pH 7.5, and tested at a final dilution of 200-fold. (B) NaDodSO$_4$/PAGE analysis of selected fractions from the C_4 chromatography shown in panel A. Half of each fraction was dried, redissolved in NaDodSO$_4$/2-mercaptoethanol, heat denatured and electrophoresed in a 14% polyacrylamide gel which was subsequently stained with silver. The position of each molecular weight marker (mass in kDa) is indicated by an arrow. (C) DNA synthesis in BALB/MK cells triggered by the fractions analyzed in Panel B. Activity is expressed as the fold stimulation over background which was 100 cpm.

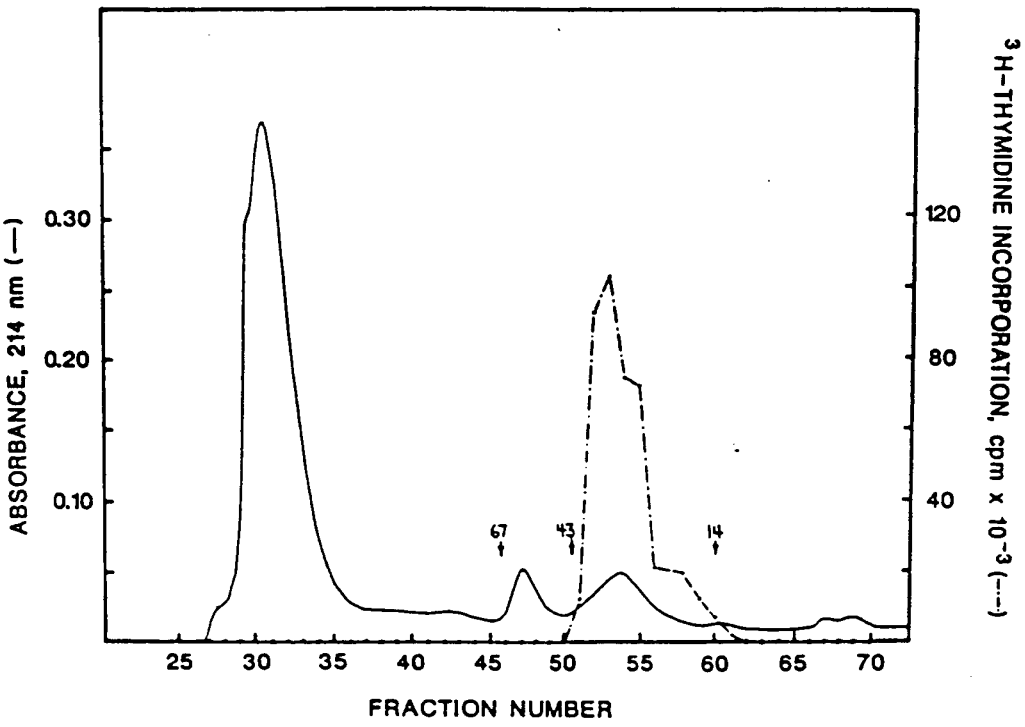


Figure I-3. Molecular sieving HPLC (TSK 3000SW) chromatography of the BALB/MK mitogenic activity. Approximately 50 µl of a Centricon-processed, 0.6M NaCl pool from HSAC were loaded onto a LKB GlasPac TSK G3000SW column (8 x 300 mm), previously equilibrated in 20 mM Tris-HCl, pH 6.8/0.5M NaCl, and eluted as 0.2 ml fractions at a flow rate of 0.4 ml/min. Aliquots of 2 µl were transferred to microtiter wells containing a final volume of 0.2 ml for assay of ^{3}H-thymidine incorporation in BALB/MK cells. The elution positions of molecular weight markers (mass in kDa) were as indicated by the arrows.

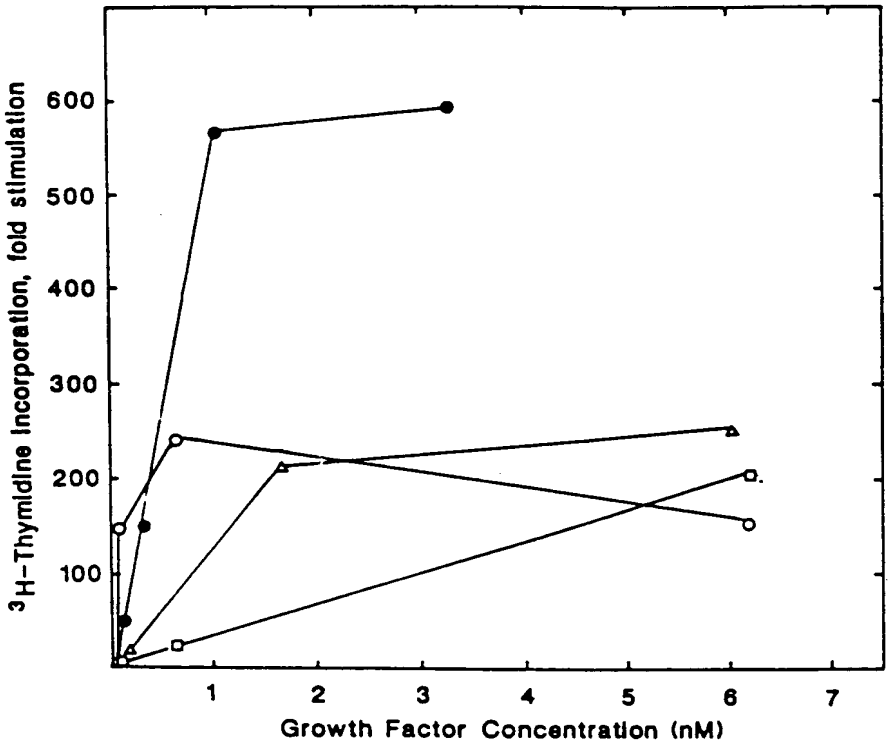


Figure I-4. Comparison of BALB/MK DNA synthesis in response to TSK-purified mitogen and other growth factors. Incorporation of ^{3}H-thymidine into trichloracetic acid-insoluble DNA, expressed as fold stimulation over background, was measured as a function of the concentration of the indicated growth factors. Background values with no sample added were 150 cpm. The results represent mean values of two independent experiments. Replicates in each experiment were within 10% of mean values. TSK-purified mitogen, •———•; EGF, Δ———Δ; aFGF, □———□; bFGF, o———o.

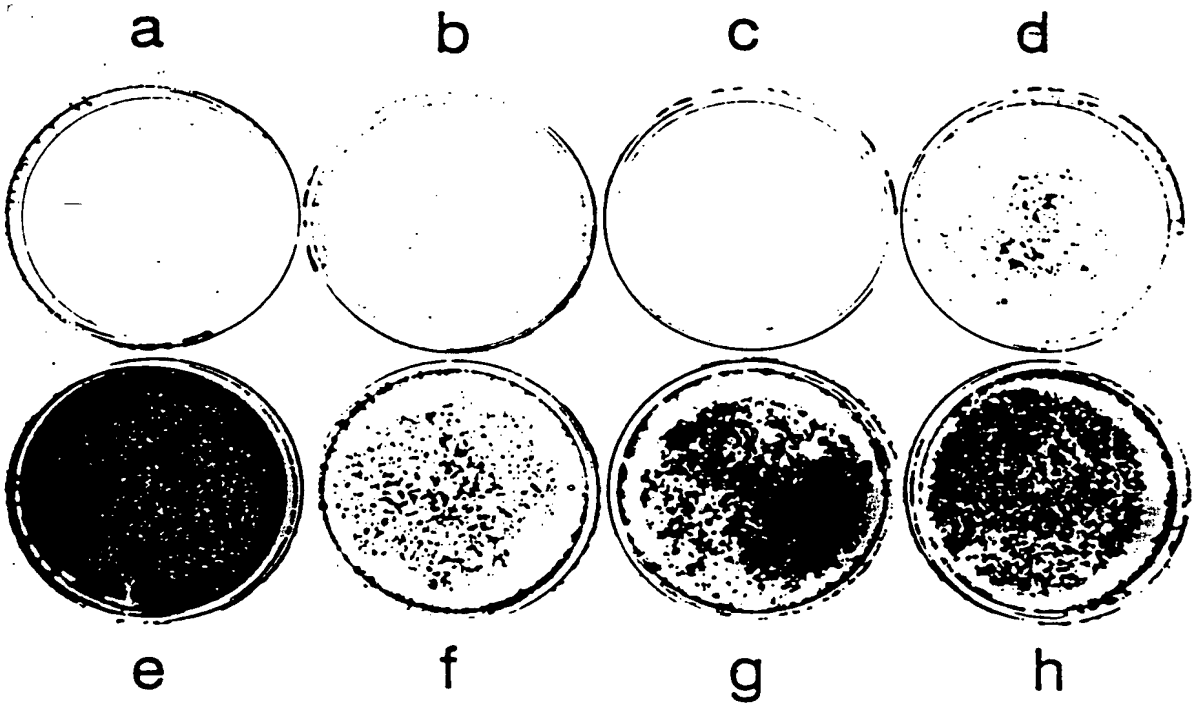


Figure I-5. Comparative growth of BALB/MK cells in a chemically defined medium in response to different combinations of growth factors. Cultures were plated at a density of 2.5×10^4 cells per dish on 35 mm Petri dishes precoated with poly-D-lysine/fibronectin in a 1:1 mixture of Eagle's minimal essential medium and Ham's F12 medium supplemented with transferrin, Na_2SeO_3, ethanolamine and the growth factors indicated below. After 10 days, the plates were fixed and stained with Giemsa. Key: a) no growth factor; b) EGF alone; c) insulin alone; d) KGF alone; ~~e) EGF + insulin~~. Final concentrations of the growth factors were as follows: EGF, 20 ng/ml; insulin, 10 μg/ml; and KGF, 40 ng/ml.

TABLE 3. Effect of Heparin on KGF Mitogenic Activity.

Growth Factor	BALB/MK		NIH/3T3	
	+	-	+	-
KGF	150	9.5	<1	<1
aFGF	106	259	10.4	68
bFGF	30	124	45.7	70

Cells were plated in microtiter plates, grown to confluence in serum containing media and then placed in a serum-free medium for 24-72 hr prior to sample addition. Mitogenesis assays were performed as described (see Experimental Section I, above and F19). Where indicated, heparin was included in the culture media at a final concentration of 20 μg/ml. The concentration of all the growth factors was 50 ng/ml. The results represent fold stimulation of ^{3}H-thymidine incorporation in the indicated assay cell in the presence (+) or absence (-) of heparin. Each value represents the mean result from two independent experiments in which each point, in turn, represents the mean value of duplicate analyses.

Figure II-1A. SEE LEGEND FOLLOWING

A.

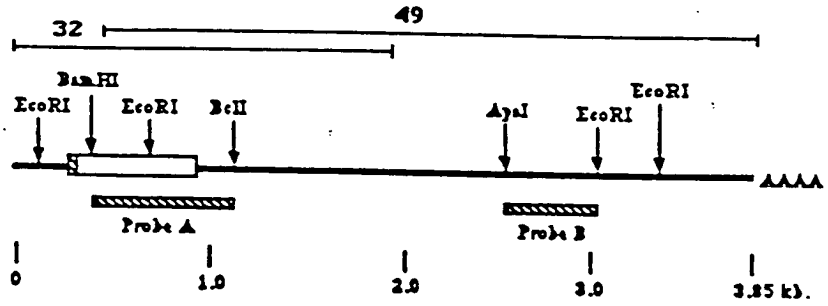


Figure II-1B. SEE LEGEND FOLLOWING

B.

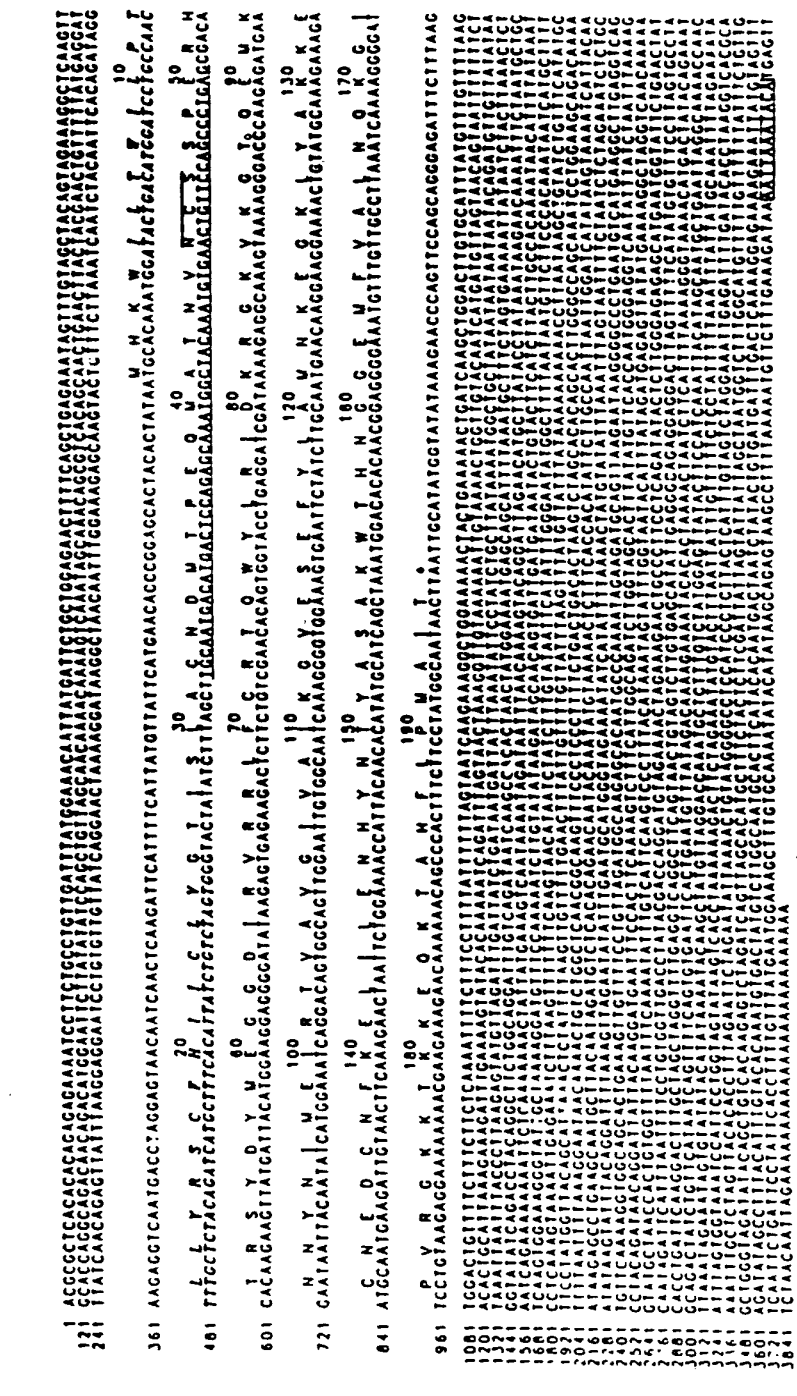
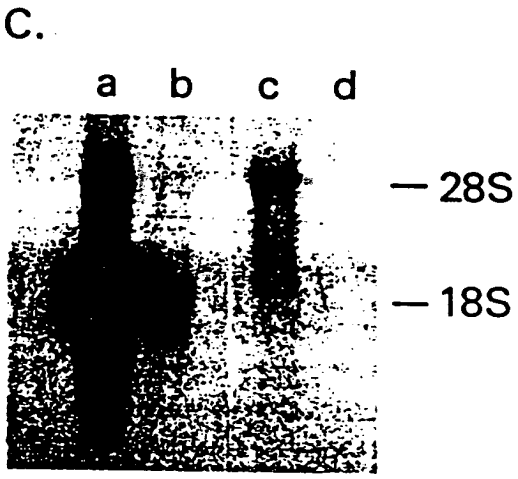


Figure II-1C. SEE LEGEND FOLLOWING



~~Figure II-1. Nucleotide sequence and deduced amino acid sequence of KGF cDNA, and identification of KGF gene transcripts.~~ Figure 6 outlines a ~~(A)~~ Schematic representation of human KGF cDNA clones. Overlapping pCEV9 clones 32 and 49, used in sequence determination, are shown above a diagram of the complete structure in which untranslated regions are depicted by a line and the coding sequence is boxed. The hatched region denotes sequences of the signal peptide ~~and the open region of the mature protein~~. Figure 7 documents the ~~(B)~~ Selected restriction sites are indicated. KGF cDNA nucleotide and predicted amino acid sequences. Nucleotides are numbered on the left ~~right~~; amino acids are numbered throughout. The N-terminal peptide sequence derived from purified KGF is underlined. The hydrophobic N-terminal domain is italicized. The potential asparagine-linked glycosylation site is overlined. The variant polyadenylation signals, AATTAA and AATACA, close to the 3' end of the RNA, are boxed. Figure 8 shows ~~(C)~~ Identification of KGF mRNAs by Northern blot analysis. Lanes a and c, poly(A)-selected M426 RNA; lanes b and d ~~lane d~~, total cellular M426 RNA. Filters were hybridized with a ^{32}P-labeled 695 bp *Bam*HI/*Bcl*I fragment from clone 32 (Probe A, Fig. ~~II-1A~~), lanes a and b,

or a 541 bp *Apa*I/*Eco*RI fragment from clone 49 (Probe B, Fig. ~~II-1A~~ 6A), lanes c and d.

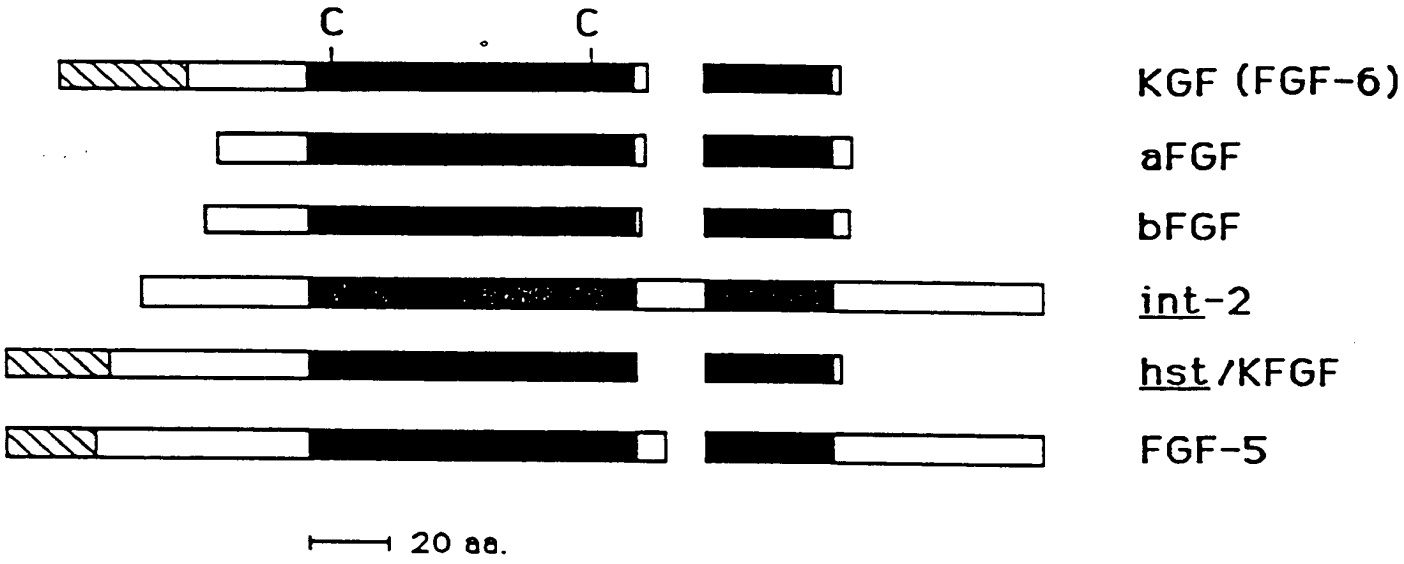


Figure II-2. Topological comparison of the FGF family of related molecules. The two protein domains that share high homology are shown by shaded boxes. Hatched boxes indicate putative signal peptide sequences. The positions of two conserved cysteine residues (C) are shown.

Figure II-3. SEE LEGEND NEXT PAGE

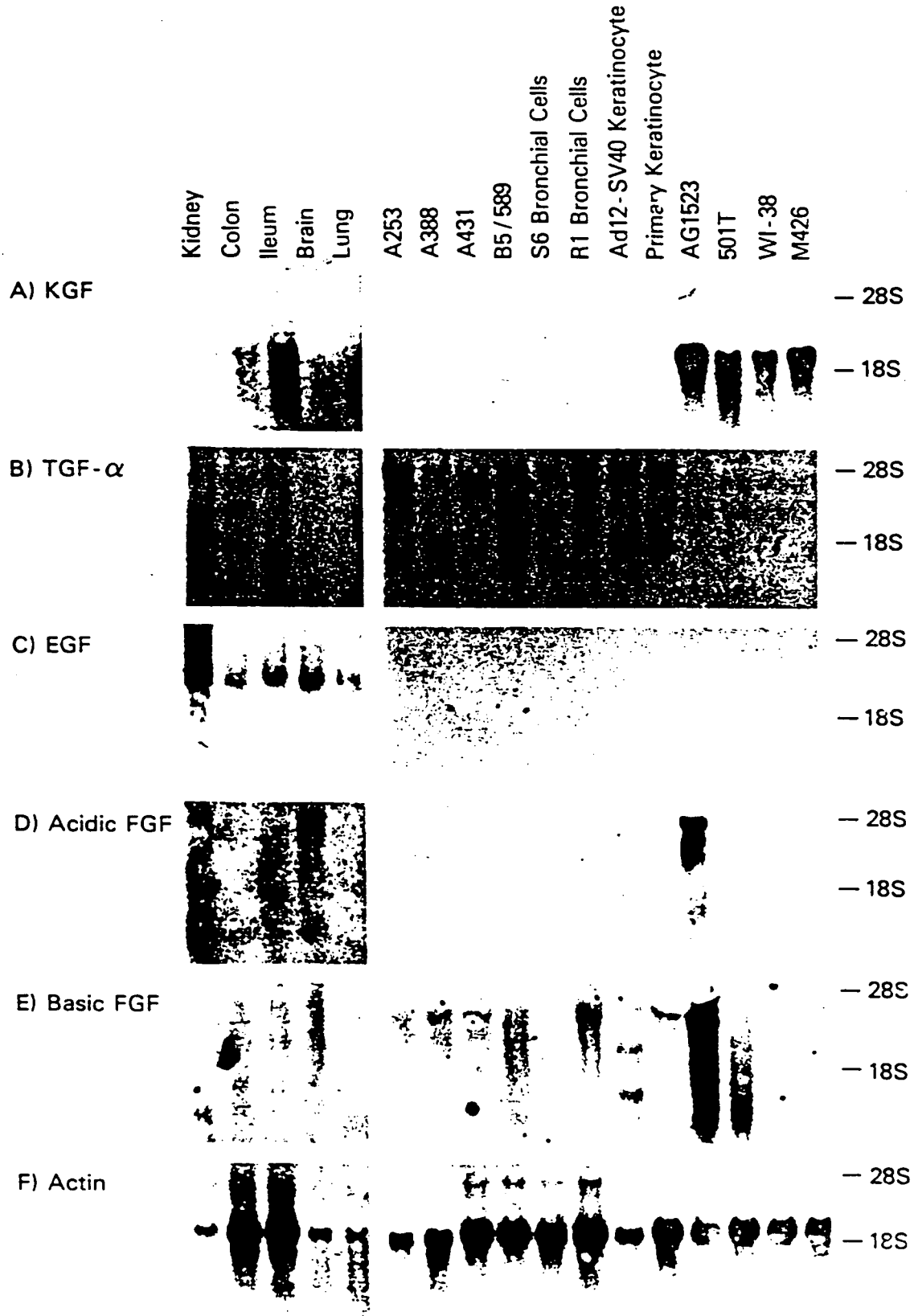


Figure II-3. Northern blot analysis of KGF mRNA in normal human cell lines and tissues, and comparison with mRNA expression of other growth factors with known activity on epithelial cells. Total cellular RNAs were isolated by cesium trifluoro-acetate gradient centrifugation. 10 µg of RNA were denatured and electrophoresed in 1% formaldehyde gels. Following mild alkali denaturation (50 mM NaOH for 30'), RNA was transferred to nitrocellulose filters using 1 M ammonium acetate as a convectant. Filters were hybridized to a ^{32}P-labelled cDNA probe containing the ~~647bp EcoRI fragment from the 5' end~~ BamHI/BclI fragment containing the majority of the KGF coding sequence (A) or similar probes from the other growth factor DNAs. The following human cell types were used: squamous cell carcinomas (A253, A388 and A431); mammary epithelial cells (S6 and R1); keratinocytes immortalized with Ad12-SV40; primary human keratinocytes; neonatal foreskin fibroblasts, (AG1523); adult skin fibroblasts (501T); and embryonic lung fibroblasts (WI-38 and M426).